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In vivo Fatty Acid Synthesis in Adipose Tissue and Liver of Meal-Fed Rats.* (32020)

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Tepperman *et al*(1) observed increased RQ values in rats restricted to a single daily meal period (meal-fed), suggesting enhanced lipid synthesis. Subsequent studies by these workers and others demonstrated significantly greater incorporation of ^{14}C-labelled substrates into fatty acids by liver slices from meal-fed as compared to slices from control rats(2). More recently meal-feeding has been shown to induce even more dramatic increases in fatty acid synthesis in rat epididymal adipose tissue(3-5).

In vitro data on the conversion of radioactive substrates to fatty acids and the original RQ measurements reported by Tepperman *et al*(1) imply that fatty acid synthesis is enhanced in the intact meal-fed rat but this has not been demonstrated. Consequently an experiment was designed to study fatty acid synthesis *in vivo* and also to obtain information on the relative importance of adipose tissue and liver as sites of fatty acid synthesis. The results of this investigation form the basis for the present report.

Methods. Male Holtzman rats weighing about 220 g were used. The animals were housed singly in metal cages having raised wire floors and in a temperature controlled room. A commercial rat diet† in powdered form was fed throughout this study.

The rats were divided into 2 equal groups on the basis of body weight. One group was fed *ad libitum* (nibblers) and the others had access to food from 8 A.M. to 10 A.M. only (meal-eaters). The animals were maintained on these regimens for 2 weeks, a period shown to be adequate for induction of enhanced lipogenesis and the accompanying enzymatic changes(6). Water was available at all times and food consumption and body weights were determined at weekly intervals. The nibbling animals were fasted from 10 A.M. on the day preceding the experiment until 8 A.M. on the day of the experiment as were the meal-fed animals. At this time both meal-fed and nibbling animals were given 10 g of food, an amount which all animals rapidly consumed. At the start of the feeding period each animal received 4.5 μc of glucose-U-^{14}C in 0.1 ml of physiological saline intraperitoneally. The radioactive glucose had a specific activity of 3.46 μc/μmole and consequently an insignificant amount of glucose was given (234 μg per rat). Four rats from each group were killed 3, 6 and 9 hours after the start of the meal period. The animals were decapitated, blood was collected and the necessary tissues were rapidly removed and weighed. The epididymal adipose tissue was placed in chloroform:methanol (2:1, v:v) to extract the lipids, liver lipids were extracted with the same solvent. The lipid extracts were washed by the method of Folch *et al* (7) to remove non-lipid radioactive materials and the solvent was evaporated. The residual lipid was saponified by refluxing with 10 ml of 5% ethanolic KOH, an equal volume of water was then added and the non-saponifiable lipids were removed by three successive extractions with petroleum ether (BP-30-60°). The aqueous phase was acidified with HCl and the fatty acids were removed by repeated extraction with petroleum ether. The ether extracts were combined in liquid scintillation vials, the solvent was evaporated

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† Rockland mouse/rat diet (complete), Teklad, Inc., Monmouth, Ill.

TABLE I. Incorporation of Glucose-U-^{14}C into Fatty Acids of Adipose Tissue, Liver and Serum Lipids in Meal-Fed and Nibbling Rats*.

Time after initiation of feeding (hr)	Regimen	Glucose-U-^{14}C incorporated into fatty acids		
		Adipose tissue (dpm × 10^3/g)	Liver (dpm × 10^3/g)	Serum (dpm × 10^3/100 ml)
3	Meal-eating	40.92 ± 6.74†	.72 ± .26	4.43 ± 1.20
	Nibbling	.18 ± .06	.08 ± .01	3.57 ± 1.18
	P‡	<.01	<.05	ns
6	Meal-eating	38.22 ± 4.24	.75 ± .30	4.56 ± .74
	Nibbling	.50 ± .06	.14 ± .03	1.98 ± .20
	P	<.01	ns	<.01
9	Meal-eating	79.63 ±21.10	2.90 ± .78	10.87 ± 1.26
	Nibbling	.37 ± .11	.13 ± .03	2.54 ± .58
	P	<.02	<.02	<.01

* All rats were fasted for 22 hr prior to experiment. At initiation of experiment each rat received 4.5 μc of glucose-U-^{14}C (3.46 μc/μmole) intraperitoneally and was fed 10 g of diet. The animals were then killed at times indicated. Values for adipose tissue and liver expressed on a wet weight basis.

† Mean for 4 rats ± standard error.

‡ P = probability of differences being significant; ns = not significant.

and the fatty acids were dissolved in 10 ml of toluene scintillant(5). Serum fatty acids were isolated in the same way except that serum lipids were saponified without prior extraction.

Serum glucose was determined by the glucose oxidase procedure using Glucostat‡ reagents. Serum cholesterol and liver total lipids and cholesterol were determined as previously described(8,9). Serum total lipids were determined by the method of Huerga *et al*(10). The data were evaluated statistically by means of the "t" test.

Results and discussion. The recovery of radioactivity in adipose tissue, liver and serum fatty acids is shown in Table I. During the early periods following the initiation of the meal a considerable amount of glucose of dietary origin is absorbed and presumably fatty acid release from liver and adipose tissue is minimal. Under these conditions the radioactivity in liver and adipose tissue fatty acids undoubtedly represents conversion of glucose-^{14}C to fatty acids in these tissues(11). The data in Table I show that 3 hours after the start of the meal period, meal-fed rats had incorporated about 200 times more glucose into adipose tissue fatty acids than had nibbling rats and in liver the difference was about 9 times greater for the meal-fed animals. The assumption that the radioactivity in adipose tissue and liver fatty acids represents synthesis in these tissues is supported by the observation that 6 hours after initiation of the meal the fatty acid radioactivity in these tissues remained essentially unchanged. The radioactivity in serum fatty acids was similar for meal-fed and nibbling rats 3 hours after the start of feeding and in meal-fed animals had not changed after 6 hours but decreased in serum of the nibbling rats. The reason for this decrease in nibbling animals is not apparent.

The radioactivity in adipose tissue, liver and serum fatty acids of meal-fed rats increased markedly in the period between 6 and 9 hours after the start of the meal. The source of this radioactivity is not known but could have been derived from muscle and adipose tissue glycogen. Muscle of meal-fed rats accumulates substantial quantities of glycogen during the meal period(6), which could serve as an energy source for this tissue in the transition period after the meal when dietary glucose becomes limiting and before fatty acids are available for oxidation. The metabolism of glucose derived from glycogen by muscle would yield substantial quantities of lactate(12) which can serve as a substrate for fatty acid synthesis in adipose tissue. Adipose tissue of meal-fed rats also

‡ Glucostat®, a prepared enzmatic glucose reagent, Worthington Biochemical Corp., Freehold, N. J.

TABLE II. Serum Glucose and Serum and Liver Total Lipid and Cholesterol Levels in Meal-Fed and Nibbling at Varying Times After Feeding.*

Time after initiation of feeding (hr)	Regimen	Serum			Liver	
		Glucose (mg/100 ml)	Total lipids (mg/100 ml)	Cholesterol (mg/100 ml)	Total lipids (%)	Cholesterol (mg/g)
3	Meal-eating	145 ± 3†	212 ± 7	55 ± 0	5.5 ± .1	3.40 ± .04
	Nibbling	160 ± 7	241 ± 22	64 ± 4	5.4 ± .1	3.28 ± .04
	P‡	ns	ns	ns	ns	ns
6	Meal-eating	124 ± 4	232 ± 10	60 ± 1	5.0 ± .2	3.02 ± .11
	Nibbling	147 ± 6	242 ± 14	62 ± 4	5.3 ± .2	3.45 ± .08
	P	<.02	ns	ns	ns	<.01
9	Meal-eating	133 ± 4	270 ± 19	60 ± 3	5.2 ± .1	2.85 ± .05
	Nibbling	142 ± 5	224 ± 13	57 ± 2	4.8 ± .2	3.17 ± .06
	P	ns	ns	ns	<.05	<.01

* Experimental conditions as indicated in Table I. Liver values expressed on a wet weight basis.

† Mean for 4 rats ± standard deviation.

‡ Probability of differences being significant; ns = not significant.

accumulates substantial quantities of glycogen(6) which might be used as a source of carbon for fatty acid synthesis. Consequently glycogen could be the source of the increased radioactivity observed in adipose tissue fatty acids between 6 and 9 hours after the start of the meal-period (Table I). The increase in serum fatty acid-^{14}C of meal-fed rats during this same period may well be the result of an increased release of fatty acids from adipose tissue. Uptake of these acids by liver could account for the observed increase in liver fatty acid radioactivity between 6 and 9 hours after initiation of the meal. The data presented in Table I clearly show that fatty acid synthesis *in vivo*, is markedly enhanced in liver and adipose tissue of meal-fed rats, a finding in accord with *in vitro* studies(2-5). The changes which occur at about 9 hours after the start of the meal period are not explained by the results of this investigation. However, the explanation proposed above is consistent with observations of glycogen synthesis in muscle and adipose tissue of meal-fed rats(6) and with our present understanding of adipose tissue metabolism.

The concentrations of glucose, total lipids and cholesterol in serum were determined as well as the levels of total lipids and cholesterol in liver. These results are shown in Table II and generally there was little influence of meal-feeding on these parameters. The observations on serum and liver lipids and cholesterol are in general agreement with the observations of Okey *et al*(13).

The results of this study permit an estimate of the relative importance of liver and adipose tissue as sites of fatty acid synthesis in the intact meal-fed rat. This has been estimated using the incorporation rates shown in Table I for animals sacrificed 3 hours after the start of the meal period, values presented by Shaffir and Wertheimer(14) on body fat content for rats of this size (total body fat = 11% of body weight; epididymal fat = 12% of total body fat) and liver weights for these animals. In the meal-fed rat whether it is assumed that the observed incorporation of glucose into fatty acids applies to epididymal adipose tissue alone or to the total body adipose tissue, at least 95% of the fatty acids are apparently synthesized in adipose tissue. In the nibbling animals, assuming that all body adipose tissue synthesized fatty acids at the rate observed for epididymal adipose tissue, 89% of the fatty acids appear to be derived from adipose tissue. This value is undoubtedly high since not all adipose tissue synthesizes fatty acids at the same rate(14). At the other extreme, by disregarding all but epididymal adipose tissue, about 50% of the fatty acids are synthesized by this tissue. However, this is obviously an underestimate since adipose tissue from other sites does have a lipogenic capacity(14). From these estimates, it would seem fair to conclude

that in the nibbling rat adipose tissue accounts for from 50 to 90% of the total fatty acids synthesized whereas in the meal-fed rat, where lipogenesis is markedly enhanced, adipose tissue accounts for at least 95% of the total fatty acid synthesis. These estimated values are in general agreement with the conclusions of Favarger(11) for the mouse. Although these values are merely estimates, they do suggest that adipose tissue is the major site of fatty acid synthesis in the intact rat particularly under conditions of enhanced lipogenesis, such as in the meal-fed animals.

Summary. The results of these studies show that lipogenesis is enhanced in liver and adipose tissue of intact rats as a consequence of meal-feeding. Fatty acid synthesis was approximately 200-fold higher in adipose tissue and 9-fold higher in liver of meal-fed as compared to nibbling rats. Increases in fatty acid radioactivity in adipose tissue, liver and serum were observed between 6 and 9 hours after the initiation of the meal. A possible explanation for these changes is proposed. Serum glucose and serum liver total lipid and cholesterol levels were also determined and no significant alterations due to meal-feeding were noted. From the observed rates of glucose-U-^{14}C incorporation into liver and adipose tissue fatty acids an estimate was made of the relative importance of these tissues as sites of fatty acid synthesis. These calculations suggest that in the nibbling rat 50-90% of the fatty acids are synthesized in adipose tissue whereas when fatty acid synthesis is stimulated by meal-feeding, adipose tissue apparently accounts for about 95% of the total fatty acids synthesized.

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